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urpose: To try another miniprep - 10.5 Kb fragment contained in pDELTA1 - and amplify

used overnight cultures grown in the presence of Tet + Kan stored at 4°, once O/N incub.

Alkaline lysis protocol. all resuspended in 25 µl of TE
↑ extracted from 3 x 1.5 ml culture

Did an enzyme titration }
± Mg " } - amplification done only with miniprep DNA. no plasmids were kept. gels.

ncluded was just 1 Rx with Taq 2U

Vol 50 µl. 200 µM dNTP
4, 3, 2 mM Mg
.4 µM primer
{ 1, 2, 5 U enzyme }
{ .01 .02 .05 }

cycling 3 step
95°, 3'
(95°, 45")
(55°, 30") 25 cycles.
(72°, 5')

repared premix with buffer B containing 2 mM Mg
added supplement Mg accordingly.

2 mM	3 mM	4 mM
0	5	10
50	45	40
50	50	50

sed 2 µl of miniprepped Template
Con. unknown.

repared 20x premix

x buffer	100		
dNTP	20	4Ø x 20	
P1	4		40
P2	4		5
Temp.	40		5
enzyme	- (in 5 µl)		50
Mg	- (")		

} 5 µl /Rx as needed
enzyme 1 µl added
5 + 2 = 7 total
dil. con.

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Invented by ______ Date 1/10/95
Recorded by R. Sitaraman

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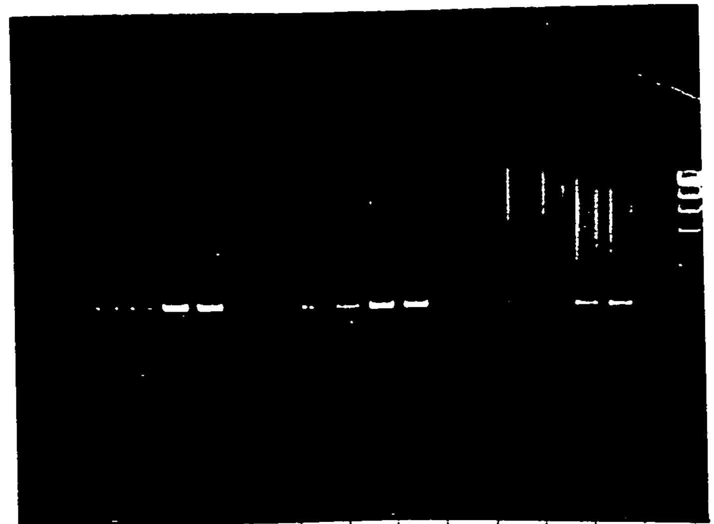
1	2	}	1U	2
3	4			3
5	6			4
7	8	}	2U	2
9	10			3
11	12			4
13	14	}	5U	2
15	16			3
17	18			4
19	Taq		2U	2 mM

1   2   5

2 3 4   2 3 4   2 3 4



Result :

cycling has to be optimized — Lot of mispriming

2 mM Mg didn't work in any of the sets ? — It worked earlier in 25 µl Rx w Taq.

- amount of template ?
- Increasing the enzyme didn't seem to work so does Mg
- Taq alone at 2U / 50 µl didn't work.
- get fresh enzyme.

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[signature]	1/25/95	Recorded by K. Sivaraman	1/10/94